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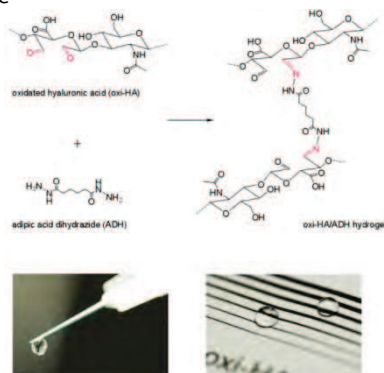
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following the ISO standard (tests for in vitro cytotoxicity), and the hydrogel was found to be non-toxic. In a preliminary animal study, the oxi-HA/ADH hydrogel was injected into the vitreous cavity of rabbit eyes. The evaluations of slit-lamp observation, intraocular pressure, cornea thickness, electroretinography (ERG) and histological examination showed no significant abnormal biological reactions for 4 weeks.

**Conclusions.** This study suggests that the injectable oxi-HA/ADH hydrogel should be a potential vitreous substitute.

**Keywords.** Vitreous; Hyaluronan; Injectable; Vitreous substitute



### (39.02) FIBROIN-BASED MATERIALS SUPPORT CO-CULTIVATION OF LIMBAL EPITHELIAL AND STROMAL CELLS

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**Introduction.** The silk protein fibroin (*Bombyx mori*) provides a potential substrate for use in ocular tissue reconstruction. We have previously demonstrated that transparent membranes produced from fibroin support cultivation of human limbal epithelial (HLE) cells (Tissue Eng A. 14(2008)1203-11). We extend this body of work to studies of human limbal stromal cell (HLS) growth on fibroin in the presence and absence of serum. Also, we investigate the ability to produce a bi-layered composite scaffold of fibroin with an upper HLE layer and lower HLS layer.

**Materials and methods.** Primary cultures of HLE and HLS cells were established in DMEM/F12 medium with 10% fetal bovine serum (FBS). Cultures were subsequently passaged onto transparent fibroin membranes or within 3D scaffolds prepared from partially-solubilised fibroin. Primary cultures of HLE and HLS cells were also established separately in serum supplemented media and cultured together upon bi-layered silk fibroin or single-layered amniotic membrane (gold standard). Tissue constructs were paraffin-embedded and analysed via immunohistochemistry.

**Results.** HLE and HLS cultures grown in 10% FBS were able to adhere to and proliferate on silk fibroin 3-D scaffolds and transparent films respectively. HLE silk constructs expressed ΔNp63+ and CK3/12+ comparably to amniotic membrane. HLE and HLS cells were able to be co-cultivated on composite fibroin scaffolds and amniotic membrane.

**Conclusions.** HLE and HLS cultures can be grown on fibroin-based materials and can be co-cultivated in a bi-layered scaffold of silk fibroin. These results encourage progression to studies of efficacy in a pre-clinical animal model.

**Keywords.** Silk fibroin; cornea; limbus; transplantation

### (39.03) OPTIMISATION OF PRIMARY CELL CULTURE CONDITIONS FOR RETINAL AND IRIS PIGMENT EPITHELIAL CELL TRANSPLANTATION ON ARTIFICIAL SUBSTRATES

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**Introduction.** A potential treatment for age-related macular degeneration (AMD) could be subretinal transplantation of a functioning retinal pigment epithelium (RPE). Iris pigment epithelial cells (IPE) have similar properties to RPE cells. They are relatively easy to obtain surgically and may be an alternative cell source for RPE replacement. We have demonstrated that surface-modified ePTFE can support a functional monolayer of an RPE cell line. The aim of this work was to investigate the ability of ePTFE membranes modified by plasma polymerisation to support the growth of a differentiated monolayer of primary human RPE cells (hRPE), primary rat and porcine IPE (rtIPE and pIPE).

**Materials and methods.** ePTFE membranes were coated with n-heptylamine (HA). For experiments with rtIPE, substrates were coated with fibronectin. hRPE and pIPE were isolated from cadaver eyes and grown for several passages. rtIPE were isolated from rat eyes and seeded directly onto substrates. Cells were seeded onto HA-ePTFE and tissue culture plastic (TCPS) substrates in a high-serum medium. Medium was replaced at 48h with a low-serum, retinoic acid-containing medium. Cells were observed by light microscopy. hRPE were stained for F-actin and markers of tight and adherens junction formation.

**Results.** Actin belts, typical of differentiated epithelial cells, formed around hRPE cells on HA-ePTFE by 14d and on TCPS by 28d. Tight and adherens junctions were uniformly distributed over all substrates by 28d. rtIPE and pIPE cells attached and spread on HA-ePTFE substrates. They were heavily pigmented and exhibited epithelial morphology (Fig. 2). Melanin was distributed around cell nuclei.

**Conclusions.** Surface modification of ePTFE by plasma polymerisation can promote the attachment of IPE and formation of a differentiated layer of RPE cells. This strategy may contribute towards development of a transplantation treatment for AMD.

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**Keywords.** Retinal pigment epithelium, iris pigment epithelium, transplantation, vision